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Skin-blanching is associated with FEV₁, allergy, age and gender in asthma families

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Summary

Background: Inhaled glucocorticosteroids reduce airway inflammation in asthma patients, thereby improving lung function and reducing airway hyperresponsiveness and symptoms. The response to glucocorticosteroids can be measured with the glucocorticosteroid skin-blanching test. We investigated if asthmatics have a lower skin-blanching response to glucocorticosteroids than non-asthmatic subjects and if asthmatics with airway obstruction have lower skin-blanching response than those without obstruction. Finally, we assessed which clinical and inflammatory parameters influence the variability in skin-blanching response.

Methods: We evaluated the skin-blanching response to topical budesonide in a large group of 315 well-characterized asthmatics and their relatives (asthma $n = 114$, healthy $n = 140$, other = 61).

Results: The skin-blanching scores of the asthma probands and their healthy spouses were not significantly different. The skin-blanching score of patients with FEV₁ < 80% predicted was lower than of patients without obstruction. Lower skin-blanching score was significantly

Abbreviations: FEV₁, forced expiratory volume in 1 s; AMP, adenosine-5-monophosphate; MRC, Medical Research Council; IgE, immunoglobulin E; PC20, provocative concentration causing a 20% fall in FEV₁; PD20, provocative cumulative dose causing a 20% fall in FEV₁; OCT3, organic cation transporter 3; GR, glucocorticoid receptor; GRE, glucocorticosteroid responsive element.

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associated with lower FEV₁ %predicted, higher age, female gender, absence of allergy and summer season, but not with use of inhaled or oral glucocorticosteroids or packyears smoking. *Conclusions:* Asthmatics do not have lower skin-blanching response to glucocorticosteroids than healthy subjects. Furthermore, lower skin-blanching response to glucocorticosteroids is associated with lower FEV₁, female gender, higher age and the absence of allergy. Crown Copyright © 2012 Published by Elsevier Ltd. All rights reserved.

Introduction

Asthma is a common respiratory disease, characterized by airway hyperresponsiveness and accompanied by intermittent airway obstruction and respiratory symptoms. The underlying inflammatory process in asthma is effectively treated by inhaled glucocorticosteroids.^{1–3} However, a subset of asthma patients has a reduced response to glucocorticosteroids. This reduced response can lead to worse asthma control and lower lung function. There are even patients who are completely resistant to glucocorticosteroids. These patients account for a large percentage of the burden of asthma morbidity, which greatly increases health care costs.⁴

A way to test for responsiveness to glucocorticosteroids is the glucocorticosteroid skin-blanching test, also known as the McKenzie skin-blanching test.⁵ Thus far, only two studies have compared skin-blanching response of asthma patients to healthy controls and their results have been conflicting. In the first study, asthma patients had a lower skin-blanching response than healthy controls.⁶ In this study, the asthma patients had marked airway obstruction (mean FEV₁ 63% of predicted). In another study healthy controls and asthma patients had similar skin-blanching response.⁷ However, the latter study showed that a subgroup of glucocorticosteroid resistant asthma patients who had more severe airway obstruction demonstrated reduced skin-blanching response. Taken together, it remains unclear whether a lower skin-blanching response is associated with the presence and severity of asthma.

There is considerable variability in glucocorticosteroid response between individuals,⁸ and it is unclear which variables determine the variation of the skin-blanching response to glucocorticosteroids between individuals. In this study we investigated the skin-blanching response in patients with asthma (probands) and their families. Our primary research question was whether asthma patients have a lower skin-blanching response to glucocorticosteroids than non-asthmatic subjects. Secondary, we investigated if asthma patients with airway obstruction have a lower skin-blanching response than asthma patients without airway obstruction and, which clinical and inflammatory parameters are associated with the variability in skin-blanching response.

Methods and materials

Study population

Between 1962 and 1975, asthma patient in adolescence or early adulthood were admitted to a local asthma referral

center.⁹ From this population, asthma patients were included, based on the following criteria: age <45 years, bronchial hyperresponsiveness to histamine and clinical symptoms of asthma according to current criteria of the American Thoracic Society. This cohort of asthma patients, also called probands, has been followed for 25–30 years and participated with their family members (spouse, children, children's spouses and grandchildren) in a family study on the genetics of asthma between 1991 and 1994. The first 93 families ($n = 499$) were asked to participate in a second evaluation between 1997 and 1999, which included the glucocorticosteroid skin-blanching test. The Medical Ethics Committee of the University Hospital Groningen approved the study; all participants provided written informed consent. For subjects <18 years written informed consent was provided by a parent/guardian.

Clinical evaluation

Phenotyping protocol family visit 1991–1994

Data on respiratory symptoms, allergic status, use of (glucocorticosteroid) medication and smoking were obtained by a modified version of the British Medical Research Council (MRC) questionnaire.¹⁰ For children younger than 16 years of age, the mother was asked to complete an extended respiratory symptom questionnaire. The forced expiratory volume in 1 s (FEV₁) was measured using a water-sealed spirometer (Lode Spirograph type DL, Lode b.v., Groningen, The Netherlands). A subject was considered hyperresponsive to histamine if the provocative concentration producing a 20% fall (PC₂₀ histamine) in FEV₁ was ≤ 32 mg/mL. In subjects older than 12 years intracutaneous tests with 16 common aeroallergens were performed. In children younger than 12 years, a skin prick test was performed with 10 allergens. Subjects with at least one positive skin test were considered to be allergic.

Phenotyping protocol second family visit 1997–1999

During the second family visit, questionnaires and spirometry were performed as described above. Additional phenotyping included the glucocorticosteroid skin-blanching test, measurements of blood eosinophils, blood cortisol and Immunoglobulin E (IgE) (enzyme-linked fluorescence assay, Mini Vidas, Biomerieux) and bronchial hyperresponsiveness to adenosine-5-monophosphate (AMP). A subject was considered hyperresponsive to AMP if the cumulative provocative dosage producing a 20% fall (PD₂₀ AMP) in FEV₁ was ≤ 32 mg. The glucocorticosteroid skin-blanching test was performed by application of budesonide on the skin of the volar side of the forearm using a protocol adapted from Brown et al.⁷ Briefly, budesonide was dissolved in 96%

ethanol to concentrations from 0.3 µg/mL to 1000 µg/mL (0.3, 1, 3.3, 10, 33.3, 100, 333, 1000 µg/mL). Test sites of 2 cm diameter were outlined by double-sided adhesive tape. The eight test concentrations of budesonide were randomly applied to the skin, 10 µL to each site. After evaporation of the diluent, the sites were covered with a plastic film. After 6 h of exposure the plastic film and adhesive tape were removed. The degree of skin-blanching was assessed 1 h later after resolution of any tape-related erythema. The skin-blanching score was assessed under standard lighting conditions (with no natural light) by trained observers, blinded to the distribution of concentrations. Blanching was graded according to a 7-point scale, varying from 0 (no blanching), 0.5, 1, 1.5, 2, 2.5 to 3 (intense blanching).¹¹ A pilot experiment showed high agreement between observers (weighted Kappa = 0.88, Fleiss Cohen).

Statistical methods

The presence of asthma was based on an algorithm as described previously,⁹ which incorporates bronchial hyperresponsiveness to histamine, respiratory symptoms (MRC questionnaire), smoking, airway obstruction and bronchodilator response. Subjects were divided into three categories: 'asthma', 'healthy' (no clinical evidence of asthma or COPD) and 'other' (COPD or unclassifiable airway disease). We used the skin-blanching score to assess the skin-blanching response. This score was calculated as the mean skin-blanching score over the total of all concentrations. To approximate a normal distribution, the mean skin-blanching score was log-transformed. To test for differences in skin-blanching between asthma patients and healthy subjects, we compared the skin-blanching score of asthma probands and their healthy spouses with Student's *T*-test. We used only the probands and their spouses for this analysis, since these groups are genetically independent. Sample size calculations were based on the findings of Livingston and colleagues.⁶ To detect a difference of 0.26 in the mean blanching score between probands and healthy spouses, with a standard deviation of 0.36, an alpha of 0.05 and beta of 0.9, we needed 40 subjects per group. To test which clinical and cellular variables were associated with the skin-blanching response, we performed a regression analysis for skin-blanching score. All regression analyses were performed using linear mixed models, with family as random factor. This method corrects for a possible dependency between subjects from the same family. First we examined univariate models. The pool of variables was chosen based on available literature. Variables with a *p*-value of 0.10 or less in the univariate model were entered into the multivariate analysis. Age, gender, smoking and asthma status were included in the multivariate model irrespective of the results of the univariate analyses. All analyses were performed using SPSS statistical software version 16.

Results

Members of 71 out of 93 invited families were willing to participate. These 71 families consisted of 329 individuals:

66 probands, 67 spouses, 165 children, 12 children's spouses and 19 grandchildren. The results from the skin-blanching test were available for analysis in 315 of the 329 participating subjects. It was not possible to classify one subject because of a missing bronchial hyperresponsiveness measurement. Of the 314 remaining subjects, 114 were classified as 'asthma', 140 as 'healthy' (no asthma or COPD) and 60 as 'other' (10 COPD and 50 unclassifiable). The clinical characteristics of the 315 participating individuals are outlined in Table 1.

Skin-blanching response of asthma probands and healthy spouses

The skin-blanching scores of asthma probands and healthy spouses were not significantly different ($p = 0.675$, Fig. 1). However, the skin-blanching score of asthma probands with airway obstruction ($FEV_1 < 80\%$ predicted) was lower than the skin-blanching score of asthma probands without obstruction ($p \leq 0.001$, Fig. 2). Probands with airway obstruction more often used glucocorticosteroids than probands without airway obstruction (23/32 vs. 7/31) and they were older (58 vs. 53 years) and less often allergic (23/31 vs. 30/31). Therefore, we performed regression analysis to assess which variables are associated with the skin-blanching response. Moreover, the skin-blanching response to corticosteroid was not associated with decline in FEV_1 in 44 patients with sufficient data available (data not shown).

Predictors of skin-blanching response

In the univariate analysis of all 315 subjects a lower skin-blanching score was associated with lower FEV_1 , older age, absence of allergy, the use of glucocorticosteroids, more packyears smoked and a skin-blanching test performed in summer or fall (Table 2). The skin-blanching score was not associated with gender, asthma status, bronchial hyperresponsiveness to AMP, total IgE, blood eosinophil number, cortisol, symptoms, or observer scoring the result. In the multivariate analysis (308 subjects) a lower skin-blanching score was independently associated with lower FEV_1 ($p = 0.012$), older age ($p < 0.001$), female gender ($p = 0.047$), absence of allergy ($p = 0.0019$) and a skin-blanching test performed in summer rather than winter ($p = 0.047$, Table 3).

Discussion

In this study, we provide evidence that patients with asthma do not have a lower skin-blanching response to glucocorticosteroids than non-asthmatic subjects. This is based on the observation that asthma probands and their healthy spouses had a similar skin-blanching score (Fig. 1). In addition, in our multivariate analysis asthma status was not associated with the skin-blanching score. Skin-blanching response does appear to be associated with level of airway obstruction, since probands with an $FEV_1 < 80\%$ predicted ($n = 33$) had lower skin-blanching scores than probands with normal lung function ($n = 31$). Furthermore, lower skin-blanching score was significantly associated with lower FEV_1 in the complete study population. The other main finding of this study is that

Table 1 Subject characteristics.

	All N = 315	Asthma N = 114	Healthy ^c N = 140
Age, yrs	41 (15)	46 (16)	39 (14)
Male, n (%)	164 (52)	66 (58)	73 (52)
Mean skin-blanching score	0.8 (0.5)	0.8 (0.5)	0.8 (0.4)
Never-smokers, n (%)	133 (42)	49 (43)	59 (42)
Ex-smokers, n (%)	70 (22)	31 (27)	25 (18)
Current-smokers, n (%)	111 (35)	34 (29)	55 (40)
Packyears	6.1 (9.8)	6.8 (12.2)	5.8 (8.5)
Use of glucocorticosteroids, n (%)	42 (13)	41 (35)	1 (0.7)
ICS only	37 (11)	36 (31)	1 (0.7)
ICS and oral corticosteroids	4 (1)	4 (3)	0
Oral corticosteroids only	1 (0.3)	1 (0.8)	0
Total IgE, kU/L ^a	47 (0–6120)	88 (4–6120)	27 (0–1500)
Blood eosinophils, 10 ⁷ /L ^a	9 (1–121)	12 (1–121)	8 (1–61)
Blood cortisol, nmol/L ^a	467 (38–1160)	437 (38–1121)	479 (209–1160)
Allergy ^b n (%)	161 (51)	94 (83)	40 (29)
PD ₂₀ AMP ≤32 mg, n (%)	89 (31)	60 (64)	13 (9)
FEV ₁ %predicted	96 (20)	83 (24)	105 (12)

Values are in means (SD).

^a Geometric mean (range), or number (%); FEV₁ = forced expiratory volume in 1 s.

^b ≥1 positive skin test; PD₂₀ AMP = Cumulative dose of adenosine-5-monophosphate provoking a 20% decrease in FEV₁.

^c No clinical evidence of asthma or COPD.

variability in skin-blanching response between individuals is associated with age, gender, allergy and season. Importantly, the skin-blanching score was not associated with the use of glucocorticosteroids and smoking. Although the precise mechanisms of action of the glucocorticosteroid skin-blanching test are unknown, it provides an easy, direct assessment of glucocorticosteroid sensitivity, making it an interesting test for investigating glucocorticosteroid sensitivity in asthma.

The similar skin-blanching response of asthma patients and healthy subjects is consistent with a previous study,⁷ comparing steroid-sensitive asthma patients and controls. However, it contrasts with another study in 75 asthma patients and 78 healthy controls. In this study, skin-blanching response to glucocorticosteroids was lower in asthma patients than in healthy controls.⁶ However, those asthmatics had marked airway obstruction (mean FEV₁ 63% of predicted) and this may have influenced the results, which fits with our observation that asthma probands with more severe airway obstruction (FEV₁ <80% predicted) had a lower skin-blanching response. Additionally, multivariate analysis revealed that a lower skin-blanching response was associated with lower FEV₁. These results suggest that, although skin-blanching response is similar in asthma patients in general and in non-asthmatic controls, it may be reduced in a subgroup of older patients who have non-allergic asthma and airway obstruction.

It is tempting to speculate that the airway obstruction in the subgroup of older non-allergic asthma patients with reduced skin-blanching response is associated with a lower treatment response to inhaled glucocorticosteroids, thus translating the response in the skin to the airways. However, this speculation is not supported by the results of a study in 22 patients with mild-to-moderate asthma.¹¹ In this study, the skin-blanching response to glucocorticosteroids was not associated with changes in serum cortisol or

bronchial hyperresponsiveness after a 3-week treatment with inhaled glucocorticosteroids. However, the results of this study do not rule out an effect of lower treatment response either, since included patients may have had asthma of too mild severity (mean FEV₁ was 86% predicted) to show differences in treatment response. FeNO and sputum eosinophils are validated markers of airway inflammation which can be used for assessing the response to anti-inflammatory therapy in patients with asthma^{12,13} and novel non-invasive markers of airway inflammation are being characterized.¹⁴ Future prospective studies to establish the relationships between skin-blanching response to glucocorticosteroids and lung inflammatory parameters should be undertaken.

Skin-blanching after topical application of glucocorticosteroids, is caused by sub-dermal vasoconstriction. The mechanisms of this effect may include both non-genomic (i.e. direct inhibitory effects) and genomic (i.e. transcriptional) effects through binding of the glucocorticosteroid receptor. Studies in rabbit models have provided insight into non-genomic mechanisms of glucocorticosteroid action on vasoconstriction.^{15,16} One of these mechanisms is via inhibitory effects on the enzyme organic cation transporter 3 (OCT3).¹⁷ Inhibition of OCT3 by glucocorticosteroids increases noradrenalin at the α_1 -adrenergic receptor, which then causes vasoconstriction. The rapid onset of the vasoconstrictive effect of inhaled glucocorticosteroids in the lung (i.e. 30 min) is consistent with a direct, non-genomic, effect.¹⁸ However, the maximum effect 8–12 h after application and the duration of the vasoconstrictive effect (>24 h) suggests that additional mechanisms, like genomic effects, are likely important.^{19,20} The genomic mechanisms may be binding to the glucocorticoid receptor (GR) and regulation of genes with glucocorticosteroid responsive elements (GRE's) or recruitment of histone deacetylase.²¹ An indication that regulation of genes with a GRE may be

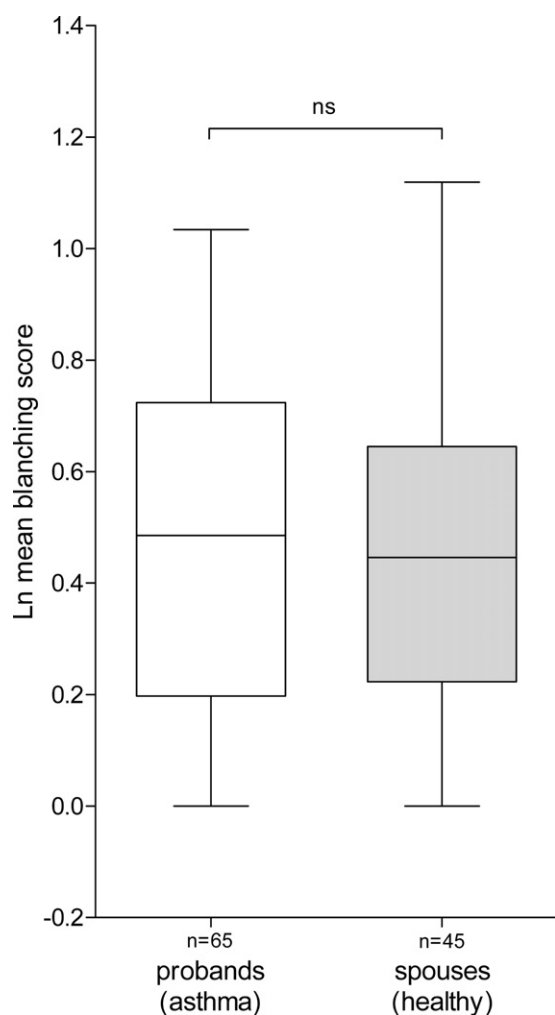


Figure 1 Skin-blanching score in asthma probands and healthy spouses. FEV₁ = forced expiratory volume in 1 s; ns = not statistically significant. Boxplot showing skin-blanching score in asthma probands and healthy spouses.

involved, is that screening of the Genomatrix database showed a putative GRE in the promoter region of the *OCT3* gene (Huge gene name: *Solute carrier 22, member 3*, *SLC22A3*).²² Further evidence for a role of GR's in skin-blanching response to glucocorticosteroids, was provided by a study of skin-blanching response in healthy volunteers with different genetic variants of the glucocorticosteroid receptor gene. A BclI restriction fragment length polymorphism (AA genotype) of the GR gene was associated with an increased skin-blanching response to glucocorticosteroids.²³ Finally, inhibition of the GR by a pharmacological inhibitor resulted in decreased skin-blanching response in healthy subjects, supporting the role of GR's in the skin-blanching response.²⁴ We found an increased skin-blanching response to glucocorticosteroids in individuals with positive skin tests to allergens, but not with elevated blood eosinophils or total serum IgE levels. This observation was surprising since asthma patients with higher levels of eosinophils or IgE have been reported to have a better glucocorticosteroid response.^{25,26} The mechanism of increased skin-blanching response in allergic subjects is unknown. OCT3, which is likely to be involved in the skin-

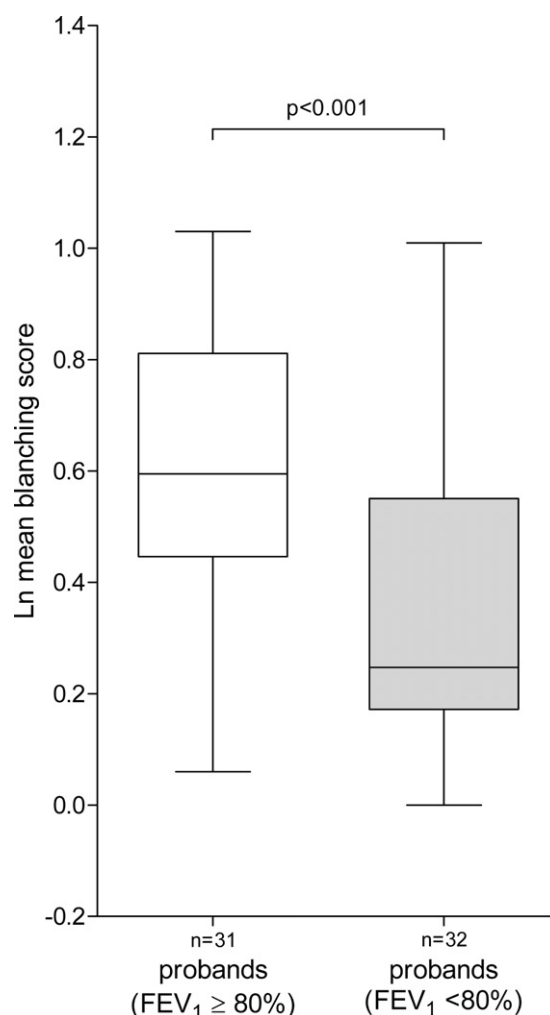


Figure 2 Skin-blanching score in asthma probands with and without obstruction. FEV₁ = forced expiratory volume in 1 s. Boxplot showing skin-blanching score in asthma probands and healthy spouses.

blanching response, may also play a role in allergy by impaired histamine clearance, since OCT3 is a transporter of histamine in rats.¹⁷ Furthermore, OCT3 mRNA was down-regulated in a rabbit model of ovalbumin-induced allergic sensitization.¹⁵

In this study, several parameters were associated with the skin-blanching response to glucocorticosteroids. First, lower skin-blanching response was associated with increasing age. Little is known about glucocorticosteroid sensitivity and age. In one study, peripheral whole blood of older men showed less inhibition by dexamethasone of IL-6 production after stimulation with lipopolysaccharide than blood of younger men, which represents lower glucocorticosteroid sensitivity.²⁷ Another study showed that the number of GR's in the hypothalamus decreases with increasing age.²⁸ However, it is not known if a similar effect is present in skin tissue. These and our studies suggest that with increasing age, glucocorticosteroid sensitivity decreases, however this has yet to be confirmed. Second, lower skin-blanching response was associated with lower lung function. No prior studies have described a relationship between glucocorticosteroid responsiveness and lung

Table 2 Univariate analysis with skin-blanching score (log transformed) as outcome variable.

Variable	N = 315		
	Univariate		
	b	SE	p-value
Age, per 10 years	-0.061	0.009	< 0.001
Gender (male vs. female)	0.038	0.028	0.177
Use of glucocorticosteroids (yes vs. no)	-0.116	0.043	< 0.007
FEV ₁ %predicted, per 10%	0.029	0.007	< 0.001
Allergy ^a	0.078	0.030	0.010
Packyears	-0.006	-0.006	< 0.001
Season			
Spring vs. winter	-0.054	0.060	0.368
Summer vs. winter	-0.166	0.061	0.007
Fall vs. winter	-0.138	0.061	0.025
PD ₂₀ AMP ≤32 mg	0.027	0.033	0.414
Total IgE (kU/L)	-0.005	0.021	0.804
Blood eosinophils (10 ⁷ /L)	0.020	0.172	0.256
Blood cortisol (nmol/L)	0.130	0.086	0.131
Symptoms (MRC)	-0.010	0.011	0.405
Observer			
Observer 1 vs. 3	0.051	0.090	0.566
Observer 2 vs. 3	0.009	0.063	0.884
Asthma status			
Asthma vs. healthy	-0.058	0.041	0.164
Unclassifiable vs. healthy	-0.035	0.040	0.379

b = regression coefficient; SE = standard error; FEV₁ = forced expiratory volume in 1 s.

Bold values represent significant p values (<0.05).

^a ≥1 positive skin test; PD₂₀ AMP = Cumulative dose of adenosine-5-monophosphate provoking a 20% decrease in FEV₁; Winter = December–February; Spring = March–May; Summer = June–August; Fall = September–November; MRC = Medical Research Council.

function in a general population. The causality of this association remains unclear. A possible explanation may be that subjects with a lower response to endogenous glucocorticosteroids have either less lung growth or a more rapid decline in lung function; however no data exists to support this claim. Third, lower skin-blanching response was associated with female gender. This finding is consistent with a study in which skin-blanching was measured with laser Doppler imaging and diffuse reflectance spectroscopy in healthy volunteers.²⁰ Gender differences were also found in a study in which asthma patients were treated with glucocorticosteroids. Treatment with inhaled corticosteroids significantly decreased the decline in FEV₁ in male, but not in female asthma patients.²⁹ These findings suggest that women have a reduced response to glucocorticosteroids. Female sex hormones may be involved in this reduced responsiveness, since estrogen has pro-inflammatory^{30,31} and progesterone anti-inflammatory properties.^{32,33} Finally, we found that the skin-blanching response was higher in winter compared to summer. This is in accordance with another study, which found the same association in healthy controls.³⁴ A possible explanation for the increased skin-blanching response is that vitamin D levels are

Table 3 Multivariate analysis with skin-blanching score (log transformed) as outcome variable.

Variable	Multivariate		
	b	SE	p-value
Age, per 10 years	-0.047	0.011	< 0.001
Gender (male vs. female)	0.055	0.028	0.047
Use of glucocorticosteroids	0.007	0.053	0.897
FEV ₁ %predicted, per 10%	0.024	0.009	0.012
Allergy ^a	0.083	0.032	0.010
Packyears	-0.001	0.002	0.406
Season			
Spring vs. winter	-0.005	0.055	0.924
Summer vs. winter	-0.112	0.056	0.047
Fall vs. winter	-0.062	0.056	0.276
Asthma status			
Asthma vs. healthy	-0.011	0.045	0.810
Unclassifiable vs. healthy	-0.032	0.038	0.394

b = regression coefficient; SE = standard error; FEV₁ = forced expiratory volume in 1 s.

Bold values represent the significant p values (<0.05).

^a ≥1 positive skin test; Winter = December–February; Spring = March–May; Summer = June–August; Fall = September–November.

increased in summer. Higher levels of vitamin D have been associated with increased glucocorticosteroid-response and higher lung function.³⁵ Another explanation may be that differences in skin tanning influences the reading of the test.

In conclusion, we have shown that asthma patients in our study population do not have a lower skin-blanching response to glucocorticosteroids than non-asthmatic subjects. When interpreting results from skin-blanching tests, the season should be taken into account, as the skin-blanching response differs between seasons. Finally, a lower skin-blanching response to glucocorticosteroids is associated with lower FEV₁, female gender, increased age and the absence of allergy.

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Conflicts of interest statement

- Eef Telenga has no conflicts of interest to disclose.
- Maarten van den Berge has received a research grant from GlaxoSmithKline in 2009.
- Judith Vonk has no conflicts of interest to disclose.
- Hajo Jongepier has no conflicts of interest to disclose.
- Leslie Lange has no conflicts of interest to disclose.
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